Mycoplasma Discussion Topics and Action Items
VICH Working Group Meeting
Ames, Iowa, July 11-14,2000

***(Discussion topics were listed by the number of comments received on the draft guideline to be discussed at the July 2000 meeting*.)**

1. (Section 2.3.2) Discuss whether the guideline should describe the PCR method in more general terms, thus allowing the methodology and primers to vary as long as the selected method can isolate the reference organisms at their required titer, or should a detailed method be specified which would then need to be validated by each laboratory using the reference organisms.

ACTION (1) for August 2000 draft: Remove the sections having to do with PCR from the guideline since it is not a required test in any of the 3 regions and based on the information presented at the March EP meeting the technology is yet of sufficient sensitivity, specificity, and reproducibility to include in a unified guideline. Include in the guideline a suggestion to use PCR for both primary isolation and identification to gain data on its usefulness as a technique for testing vaccines, cells, and master seed viruses for Mycoplasma contamination. Include in the appendix as information a method for the assay. (Note – August 2000 draft changed [in March 2001 draft] to remove reference to PCR and appendix method from guideline, add topic to "To Be Determined" list, based on other members' understanding of action at July 2000 meeting.)

ACTION (2) for justification/implementation of final guideline:
Working Group members will reference proceedings of March 2000 EP meeting when available to justify exclusion but encouragement of PCR.

2. (Sections 2.1.1, 2.2.1, and 2.3.1) Establish a method for using the Mycoplasma references, produced by the European Pharmacopoeia, to validate each laboratory's; culture method, DNA stain method, and PCR method.

ACTION (1) for August 2000 draft: Separate the guideline assay method from the specific use of the EDQM Mycoplasma references. Generalize language in guideline so as not exclude other possible options for arriving at internationally validated references should that become necessary. Include in the guideline a reference to the method of qualifying laboratories but put the currently proposed method in the Appendices. Working Group will continue to develop the method of using the EDQM Mycoplasma references using data from European laboratories' testing of the references. Remove from the guideline the section involving the qualification of the PCR method.

ACTION (2) for justification/implementation of final guideline: In preparation for distribution of these Mycoplasma reference cultures, Dr. Peter Castle is to supply the isolation and passage histories of the 5 cultures to the other countries so they can then apply for import permits and see if any problems with further distribution will be encountered.

a. (Section 2.1.1) Should the WG consider substituting, *M. gallisepticum* or *M. fermentans* for one of the other selected reference strains? Should the Mycoplasma used to detect antibiotic inhibition be changed from *A. laidlawii* to *M. Orale?*

ACTION for August 2000 draft: It was decided to include *M. fermentans* as the fifth reference organism because of the long time period required to initiate growth. *M. gallisepticum* will not be added to the list of reference organisms. Both *M. orale* and *A. laidlawii* will be used to detect antibiotic inhibition. A table will be added to the guideline to clarify when each of the reference organisms is to be used to qualify a test method.

b. (Section 2.1.1) Adopt the proposed definition of "passage"; (one transfer followed by the normally used incubation period).

ACTION for August 2000 draft: Adopt the proposed definition of "passage".

c. (Section 2.1.1) Should the working group make the phrase "approximately 100 CFU" more specific by specifying an acceptable range of 70-130 CFU.

ACTION for August 2000 draft: Adopt "approximately 100 CFU (70-130 CFU)".

3. (Section 2.1.5.1) Should the working group make any of the suggested changes to the broth and agar test method.

a. Inoculate 2 or 5 plates (0.2 ml each) on each subculture date.

ACTION for August 2000 draft: Leave the guideline as it is, requiring just one plate on each subculture but if more plates are done, all must be looked at and included in the test conclusion.

b. Number of days of incubation for broth and agar: 21 days for the broth, 10 and 14 days for the agar incubation, and inoculate the last plate on day 21 and incubate for 7 days.

ACTION (1) for August 2000 draft: Change the guideline to allow the reading of plates any time between 10 and 14 days. The day 10 subculture was deleted but a subculture on day 21 was added. The day 21 subculture plate will be read on day 28 after 7 days of incubation.

ACTION (2) for justification/implementation of final guideline: There was a question whether 7 days of incubation for the agar plate inoculated at day 21 was sufficient time to allow the slow growing *M. fermentans* colonies to grow to a large enough size for stereoscope visualization. Dr. Vannier and Dr. Castle are to provide reprints and/or written confirmation of this.

C. Adjusting pH: when is it necessary, how do you tell it is necessary, how do you do the adjustment?

ACTION for August 2000 draft: Add to the guideline that the pH of the media shall be adjusted with either NaOH or HCL, if after the addition of the sample the color of media pH indicator changes. The amount of either acid or base that needs to be added should be established at the time of prelicense. The media should be returned to its original color.

4. (Section 2.2) Should the guideline explain the relationship between the 3 test methods mentioned in this section? If the guideline allows the use of all three methods the WG must decide which methods can be used by themselves to detect Mycoplasma contamination and which must be confirmed by a second method.

ACTION for August 2000 draft: The guideline should be changed to reflect that the culture method and the DNA stain method are stand-alone methods and do not need to be confirmed by another method. The PCR method will not be included in the guideline as a required method.

5. (Section 2.1.2) The working group needs to decide whether the guideline should specify how each batch of new media is to be tested, or should the guideline just specify that the batches need to be tested, by a method acceptable to the regional authority.

ACTION for August 2000 draft: Include in the guideline that each lot of media must be tested. Include in the Appendices the suggested number and specific organisms to be used in this testing.

6. (Section 2.1) Decide on what part or parts of the production process needs to be monitored for Mycoplasma contamination; master seed virus, master cell stock; working seeds, each passage, bulk virus production before inactivation, final live virus products.

ACTION (1) for August 2000 draft: The DNA stain method will only be required when testing cell lines and master seed viruses. The culture method will be required when testing cell lines, master seed viruses, ingredients of animal origin, and final products. It was not decided whether in-process testing the working seed or cell stocks, or bulk harvest material would be required.

ACTION (2) for next discussion draft after August 2000: It was proposed to make a table containing the 2 test methods on one axis and the parts of the vaccine production process on the other axis. This table was an attachment with the e-mail for the August 2000 draft guideline. Each region was to fill in the table with their present requirements for testing, and return it to the topic leader along with any other comments. This was to be used to start the discussion on what production steps should be tested.

7. (Section 2.1.4) What should the working group adopt as the an indicator of inhibitory substances. We suggest using, a greater than 30% reduction, to make this section similar to the acceptable range in the topic above.

ACTION for August 2000 draft: Change the guideline to read that both *A. laidlawii* and *M. orale* must be isolated from broth culture containing 10 ml of the sample.

8. (Section 2.1.4) Should this guideline specify at what intervals the inhibitory substances testing needs to be repeated or should this be left up to the individual regional regulatory authority.

ACTION for August 2000 draft: Include in the guideline that the inhibitory effects of the product must be determined at the time of prelicense and whenever the formulation of the product changes.

9. (Section 2.1.6) Discuss and decide whether "retesting" is needed and if so what specifics for retesting should be included in the guideline.

ACTION for August 2000 draft: Change the section on "retesting" to allow (require?) retests only when the controls are unsatisfactory, either when the positive controls didn't grow or when the negative controls contain Mycoplasma.

10. (Section 2.2.3.1) Discuss whether specifics for the DNA staining procedure, such as cell seeding density or the length of incubation, need to be specified if growth of the reference organism can be demonstrated by the method used.

ACTION for August draft: Make the guideline require the use of VERO cells. Cite the cell density as an example but require that the first passage grow to confluence and the second passage be allowed to grow only until there are 10-15 VERO cells per field or approximately 50% confluence. A 1 ml inoculum of sample will be specified. The growth of each passage must for at least 3 days. Two reference organisms will be specified, *M. hyorhinis* and *M. orale*.

General on draft guideline:

Action for next discussion draft after August 2000: Working Group will comment on the following comments raised prior to July meeting, but not discussed at that meeting (priority given to those topics with multiple comments).

a. **1.4 (How) Should Working Stock testing be allowed to substitute for rare (old, short-supply) Master Seeds?**
b. **2.3.3 (How) Should the (minimum required) single reference for each test session be rotated/varied session to session?**
c. **2.3.5 (How) Should the agar plate colonies be confirmed as mycoplasma through staining?**
d. **2.4.1 Should "absence" be removed from the phrase "presence and absence of neutralizing antiserum" (because without the AS, the cytopathology will interfere with the test)?**
e. **Others not listed here.**